BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classificati n 6 :
C12N 15/31, 15/62, C07K 14/33, A61K 39/08, C12N 15/86, 1/21

A1

(11) International Publication Number: **WO 97/34001**

(43) International Publicati n Date: 18 September 1997 (18.09.97)

(21) International Application Number: PCT/GB97/00660

(22) International Filing Date: 11 March 1997 (11.03.97)

(30) Priority Data:
9605222.0 12 March 1996 (12.03.96) GB

(71) Applicant *(for all designated States except US)*: THE SECRETARY OF STATE FOR DEFENCE IN HER BRITANNIC MAJESTY'S GOVERNMENT OF THE UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND [GB/GB]; Defence Evaluation & Research Agency, Ively Road, Farnborough, Hampshire GU14 0LX (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: TITBALL, Richard, William [GB/GB]; C.B.D.E, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). WILLIAMSON, Ethel, Diane [GB/GB]; C.B.D.E, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). HAVARD, Helen, Louise [GB/GB]; C.B.D.E, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). OYSTON, Petra, Claire, Farquhar [GB/GB]; C.B.D.E, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB). PAYNE, Dean, William [GB/GB]; C.B.D.E, Porton Down, Salisbury, Wiltshire SP4 0JQ (GB).

(74) Agent: SKELTON, Stephen, Richard; D/IPR (DERA) F rmalities, Poplar 2a, MOD (PE) Abbey Wood #19, P.O. Box 702, Bristol BS12 7DU (GB).

(81) Designated States: AU, CA, GB, JP, NZ, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: CLOSTRIDIUM PERFRINGENS VACCINES

(57) Abstract

The present invention provides proteins for use in vaccines which are capable of inducing protective antibodies directed against C. perfringens epsilon toxin when administered to animals or man and thereby providing prophylaxis or therapy against infection by C. perfringens epsilon toxin. Particularly the present invention provides proteins which are based upon the mature toxin of the clostridium perfringens epsilon toxin gene, but which have a mutation such that the amino acid at position 106 is different to the wild-type sequence and their use in vaccine compositions.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Larvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

Clostridium Perfringens Vaccines

The present invention relates to novel peptides capable of eliciting an immunological response that is protective against Clostridium perfringens epsilon toxin in man or animals. It relates to the production of these peptides and to pharmaceutical compositions containing them, Preferred agents enable prophylaxis and treatment of Clostridium perfringens induced disease states in both humans and other animals.

Clostridium perfringens (C. perfringens) is ubiquitous in the environment and has been found in the soil, decaying organic matter and as part of the gut flora in man and animals. Different strains of C. perfringens can be assigned to one of five biotypes (A-E) depending on the spectrum of types produced see *McDonel, J.L.* (1986); *Toxins of Clostridium perfringens types A,B,C,D and E. In Pharmacology of Bacterial Toxins; F. Dorner and J. Drews, eds. (Oxford: Pergamon Press), pp. 477-517.* The epsilon toxin is produced by C. perfringens types B and D but not by types A, C or E see *Brooks, M.E., Sterne, M., and Warrack, G.H. (1957); A reassessment of the criteria used for type differentiation of Clostridia perfringens. J. Pathol. Bacteriol. 74, 185-195.* C. perfringens types B and D have a limited host range being mainly isolated from goats and cattle and rarely from man, *Smith, L.D. and Williams, B.L. (1984); The pathogenic anaerobic bacteria (Springfield, Illinois: Charles C. Thomas).* They are responsible for producing severe and rapidly fatal enterotoxaemia: C. perfringens type B enterotoxaemia infection of lambs causes lamb dysentery while type D enterotoxaemia produces pulpy kidney disease in sheep and lambs. Mortality rates in both cases may be as high as 100%. Neither disease is infectious, but sporadic outbreaks occur when the microbial balance of the gut is disrupted, for example after antibiotic treatment or due to changes in diet. Pulpy kidney disease is often associated with a change from a poor to a rich diet accompanied by excessive over-eating, *Bullen, J.J. (1970); Role of toxins in host-parasite relationships. In Micribial toxins volume 1. S. Ajl, S. Kadis, and T.C. Montie,. eds. (New York: Academic Press), pp. 233-276.* Such over-eating causes

considerable quantities of undigested, starch-rich food to pass from th rumen into the small intestine. The nutritious anaerobic environment this produces allows the multiplication of C. perfringens resulting in up to 10^9 cfu per g of ileal contents and high concentrations of epsilon toxin *Bullen, J.J. and Scarisbrick, R. (1957); Enterotoxaemia of sheep: experimental reproduction of the disease; J. Pathol. Bacteriol. 73, 494-509.* Several vaccines exist for the prevention of C. perfringens enterotoxaemia. The vaccines are based on formaldehyde-treated cell filtrates or whole cell cultures. The vaccines confer a high degree of protection in animals *Stephen, J. and Pietrowski, R.A. (1986); Bacterial toxins (England: van Nostrand Reinhold (UK) Co. Ltd.);* however, the immunogenicity of the epsilon toxin in the preparations has been reported to be variable and a more defined and consistent vaccine is preferable. Immunity to a single epitope on the toxin has been shown to be sufficient to protect against purified epsilon toxin and C. perfringens infection, *Percival, D.A., Shuttleworth, A.D., Williamson, E.D., and Kelly, D.C. (1990), Anti-idiotypic antibody-induced protection against Clostridium perfringens type D; Infect. Immun. 58, 2487-2492.*

Epsilon toxin is produced by C. perfringens types B and D as a relatively inactive prototoxin of 311 amino acids with a molecular weight of 32,700, *Worthington, R.W. and Mulders, M.S. (1977); Physical changes in the epsilon prototoxin molecule of Clostridium perfringens during enzymatic activation; Infect. Immun. 18, 549-551.* Proteolytic cleavage of 13 or 14 basic amino acids from the amino terminal of the prototoxin results in the production of the mature toxin with a molecular weight of 31,200 *Worthington and Mulders, 1977; Hunter, S.E., Clarke, I.N., Kelly, D.C., and Titball, R.W. (1992); Cloning and nucleotide sequencing of the Clostridium perfringens epsilon-toxin gene and its expression in Escherichia coli; Infect. Immun. 60, 102-110.* Activation also results in a marked shift in pI from 8.02 (prototoxin) to either 5.36 (fully active toxin) or 5.74 (partially active toxin) and a significant change in conformation (*Worthington and Mulders, 1977; Habeeb, A.F., Lee, C.L., and Atassi, M.Z. (1973); Conformational studies on modified proteins and peptides, VII; Conformation of epsilon-prototoxin and epsilon-toxin from Clostridium perfringens; Conformational changes associated with toxicity; Biochim. Biophys. Acta 322, 245-250).* A complication is that the activation of the prototoxin seems to produce several

isoforms with a range of specific activities between that of the prototoxin and the mature toxin (*Habeeb, A.F. (1975); Studies on epsilon-prototoxin of Clostridium perfringens type D. Physicochemical and chemical properties of epsilon-prototoxin; Biochim. Biophys. Acta 412, 62-69; Worthington and Mulders, 1977)*. More recently it has been found that the toxin itself also has several isoforms (*Hunter et al., 1992)*. Thus activation of epsilon prototoxin may be a multi-step process, possibly with multiple proteolytic cleavages and post-translational modifications such as deamination and phosphorylation resulting in the production of the heterogeneous mature toxin (*Hunter et al., 1992)*.

Epsilon toxin is usually obtained from a type D strain of C. perfringens and has been purified either individually or in combination by methanol precipitation, ammonium sulphate precipitation, column chromatography, size exclusion and various forms of ion exchange chromatography (*Verwoerd, D.W. (1960); Isolation van die protoksien van Clostidium welchii type D. J. S. Afr. Vet. Med. Assoc. 31, 195-203; Habeeb, A.F. (1969); Studies on epsilon-prototoxin of Clostridium perfringens type D. I. Purification methods: evidence for multiple forms of epsilon-prototoxin; Arch. Biochem. Biophys. 130, 430-440; Worthington, R.W., Mulders, M.S., and Van Rensburg, J.J. (1973); Clostridium perfringens type D epsilon prototoxin. Some chemical, immunological and biological properties of a highly purified prototoxin; Onderstepoort. J. Vet. Res. 40, 143-149; Payne, D.W., Williamson, E.D., Havard, H., Modi, N., and Brown, J. (1994); Evaluation of a new cytotoxicity assay for Clostridium perfringens type D epsilon toxin; FEMS Microbiol. Lett. 116, 161-167)*.

Traditionally, the activity of purified epsilon toxin has been determined in mouse lethality tests (*Habeeb, A.F. (1969); Studies on epsilon-prototoxin of Clostridium perfringens type D. I. Purification methods: evidence for multiple forms of epsilon-prototoxin; Arch. Biochem. Biophys. 130, 430-440; Worthington, R.W., Mulders, M.S., and Van Rensburg, J.J. (1973); Clostridium perfringens type D epsilon prototoxin. Some chemical, immunological and biological properties of a highly purified prototoxin; Onderstepoort. J. Vet. Res. 40, 143-149)*. The mature toxin is highly toxic with an LD_{50} in mice of < 100ng when administered intravenously (*Payne, D.W., Williamson, E.D., Havard, H., Modi, N., and Brown, J. (1994); Evaluation of a new*

cytotoxicity assay for Clostridium perfringens type D epsilon toxin; FEMS Microbiol. Lett. 116, 161-167). As the basis of an alternative assay for epsilon toxin activity, it has been found that the Madin Darby Canine Kidney (MDCK) cell line was sensitive to C. perfringens type D culture filtrates (*Knight, P.A., Burnett, C., Whitaker, A.M., and Queminet, J. (1986); The titration of clostridial toxoids and antisera in cell culture; Develop. biol. Standard. 64, 129-136)*. It was demonstrated that the lethal and dermonecrotic effects of the toxin observed in rabbits and its cytopathic activity were all caused by the same entity in epsilon toxin preparations and that all three activities were valid indicators in toxin neutralisation tests (*Knight, P.A., Queminet, J., Blanchard, J.H., and Tilleray, J.H. (1990); In vitro tests for the measurement of clostridial toxins, toxoids and antisera. II. Titration of Clostridium perfringens toxins and antitoxins in cell culture; Biologicals. 18, 263-270)*. Recently, the development of a new cytotoxicity assay for the determination of the activity of C. perfringens type D epsilon toxin based on the sensitivity of the MDCK cell line has been reported (*Payne, D.W., Williamson, E.D., Havard, H., Modi, N., and Brown, J. (1994); Evaluation of a new cytotoxicity assay for Clostridium perfringens type D epsilon toxin; FEMS Microbiol. Lett. 116, 161-167)*. In four out of five samples between 15-22 ng/ml of purified epsilon toxin was sufficient to reduce the viability of MDCK cells by 50% and as little as 8 ng/ml sufficient to cause a significant reduction in the viability of the MDCK cells, Payne et al., 1994.

The etx gene encoding epsilon toxin is carried out on an episome distinct from the 3.6Mb chromosome (*Canard, B., Saint Joanis, B., and Cole, S.T. (1992); Genomic diversity and organization of virulence genes in the pathogenic anaerobe Clostridium perfringens. Mol. Microbiol. 6, 1421-1429)*. The gene has been cloned and sequences for both B and D types determined. The cloned gene etxB coded for a protein of M_r~32,981 (*Hunter, S.E., Clarke, I.N., Kelly, D.C., and Titball, R.W. (1992); Cloning and nucleotide sequencing of the Clostridium perfringens epsilon-toxin gene and its expression in Escherichia coli; Infect. Immun. 60, 102-110)*. Neither the sequenced gene or the derived protein showed homology with other proteins. Comparison of the sequences of cloned etx genes from type B and type D strains revealed two nucleotide differences in the open reading frame resulting in one amino acid substitution (*Havard, H.L., Hunter, S.E., and Titball, R.W. (1992); Comparison of the nucleotide sequence*

and development of a PCR test for the epsilon toxin gene of Clostridium perfringens type B and type D; FEMS Microbiol. Lett. 76, 77-81). The promoters for the genes were not homologous, with different putative -10 and -35 sequences. This allowed the development of epsilon-specific PCR primers to produce a system for typing B and D strains of C. perfringens. The etx promoter allowed expression of the cloned gene in E.coli *(Hunter et al., 1992)*. Epsilon toxin is preceded by a signal peptide resulting in the native protein being exported from C. perfringens and the recombinant protein accumulating in the periplasmic space of E.coli *(Hunter et al., 1992; Bullen, J.J. and Batty, I. (1956); The effect of Clostridium welchii type D culture filtrates on the permeability of the mouse intestine; J. Pathol. Bacteriol. 71, 311-323)*. The recombinant toxin expressed in E.coli was shown to have identical biochemical and biological properties to those of the native toxin.

Epsilon prototoxin produced in the gut of animals is activated by proteolytic enzymes present in intestinal fluid *(Niilo, L. (1965); Bovine enterotoxaemia.III; Factors affecting the stability of the toxins of Clostridium perfringens types A, C and D; Can. Vet. J. 6, 38-42)*. The mature toxin increases intestinal permeability and enters the blood supply *(Bullen and Batty, 1956; Bullen, J.J. (1970); Role of toxins in host-parasite relationships. In Micribial toxins volume 1. S. Ajl, S. Kadis, and T.C. Montie, eds. (New York: Academic Press), pp. 233-276; Jansen, B.C. (1967); The production of a basic immunity against pulpy kidney disease; Onderstepoort. J. Vet. Res. 34, 65-80*. The mode of action of epsilon toxin is not known, but several observations have suggested that it acts upon the central nervous system. The toxin rapidly causes a widespread disturbance in the permeability balance of the brain by disrupting vascular endothelia *(Finnie, J.W. (1984); Ultrastructural changes in the brain of mice given Clostridium perfringens type D epsilon toxin; J. Comp. Pathol. 94, 445-452; Buxton, D. (1976); Use of horseradish peroxidase to study the antagonism of Clostridium welchii (Cl. perfringens) type D epsilon toxin in mice by the formalinized epsilon prototoxin; J. Comp. Pathol. 86, 67-72)*. As degenerative changes progress, serum proteins and eventually red cells leak from the vasculature, astrocyte end feet rupture and oedema ensues *(Buxton, D. and Morgan, K.T. (1967); Studies of the lesions produced in the brains of colostrum deprived lambs by Clostidium welchii (Clostridium perfringens) type D toxin; J. Comp. Path. 86, 435-447)*. In acute

cases of epsilon toxin induced entertoxaemia characteristic lesions occur at specific sites in the brain (*Hartley, 1956; Buxton, 1976; McDonel, 1986*). Chemical modification experiments have demonstrated the importance of certain amino acid residues for the lethality of epsilon toxin. One tryptophan (*Sakurai, J. and Nagahama, M. (1985); Role of one tryptophan residue in the lethal activity of Clostridium perfringens epsilon toxin; Biochem. Biophys. Res. Commun. 128, 760-766*), one histidine (*Sakurai, J. and Nagahama, M. (1987); Carboxyl groups in Clostridium perfringens epsilon toxin; Microb. Pathog. 3, 469-474*), one tyrosine (*Sakurai, J. and Nagahama, M. (1987); The inactivation of Clostridium perfringens epsilon toxin by treatment with tetranitromethane and N-acetylimidazole; Toxicon 25, 279-284*) and three or four aspartic or glutamic acids (*Sakurai, J. and Nagahama, M. (1987); Histidine residues in Clostridium perfringens epsilon toxin; FEMS Microbiology Letters 41, 317-319*) residues were shown to be essential for the lethal effect of epsilon toxin. Eight lysine residues have also been shown to be important in activity, but are probably involved in maintaining conformation rather than being integral to an active site (*Sakurai, J. and Nagahama, M. (1986); Amino groups in Clostridium perfringens epsilon prototoxin and epsilon toxin. Microb. Pathog. 1, 417-423*).

It is an object of the present invention to provide novel polypeptides for use in vaccines which are capable of inducing protective antibodies directed against C. perfringens epsilon toxin when administered to animals or man and thereby providing prophylaxis or therapy against infection by C. perfringens epsilon toxin.

The present invention provides a polypeptide capable of producing an immune response which is protective against *Clostridium perfringens*, said polypeptide comprising an amino acid sequence which has at least 60% homology with the amino acid sequence of Clostridium perfringens epsilon toxin or an immunogenic fragment thereof, characterised in that the amino acid residue corresponding to residue 106 of the mature toxin is of an amino acid other than histidine.

Suitably, the polypeptide has an amino acid sequence which has at least 80% homology and preferably 90% homology and is most preferably substantially completely homologous with the amino acid sequence of Clostridium perfringens epsilon toxin or an immunogenic fragment thereof.

The amino acid sequence of Clostridium perfringens epsilon toxin is shown hereinafter in as amino acids 1-283 of Figure 2 (SEQ ID No 2). Where the polypeptide of the invention is homologous to that of SEQ ID No 2 or an immunogenic fragment thereof, it is preferable that any altered amino acids are replaced by conservative substitutions.

By 'conservative substitution' is meant the substitution of an amino acid by another one of the same class; the classes being as follows:

CLASS	EXAMPLES OF AMINO ACID
Nonpolar:	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged polar:	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic:	Asp, Glu
Basic:	Lys, Arg, His

As is well known to those skilled in the art, altering the primary structure of a peptide by a conservative substitution may not significantly alter the activity of that peptide because the side-chain of the amino acid which is inserted into the sequence may be able to form similar bonds and contacts as the side chain of the amino acid which has been substituted out. This is so even when the substitution is in a region which is critical in determining the peptides conformation.

Non-conservative substitutions are possible provided that these do not interupt with the immunogenicity of the polypeptide.

The expression "immunogenic fragment" used herein refers to a polypeptide which is shorter than full length native toxin, but which includes at least one antigenic determinant and also which includes a residue corresponding to residue 106 of the mature toxin. Suitably the fragments will comprise at least 15, more suitably at least 30 and preferably at least 60 amino acids.

In particular, the polypeptide comprises a protein which has an amino acid sequence which has at least 60% homology with the amino acid sequence of Clostridium perfringens epsilon toxin characterised in

that the amino acid residue corresponding to residue 106 of the mature toxin is of an amino acid other than histidine.

Most preferably the protein comprises the amino acid sequence of clostridium perfringens epsilon toxin and is characterised in that the amino acid residue corresponding to residue 106 of the mature toxin is of an amino acid other than histidine.

Preferably the amino acid at position 106 is a non-basic amino acid, and in particular a non polar amino acid, especially proline.

The polypeptides or proteins of the invention are genetically toxoided (inactivated) which means that they are less likely to cause unwanted side effects in animals to which they are administered. This is a much more precise and quantifiable way of inactivating the toxin rather than using chemical toxoiding methods.

It should also be stressed that the invention also encompasses peptides comprising the amino acid sequences described above i.e. wherein the N- or C- terminus has been extended. Extension of the peptides above may confer additional desirable properties on them, for instance, easier separation or purification, or enhancing or adding to the immunity or labelling.

In particular, the polypeptide or protein described above may form part of a fusion protein which may further comprise a moiety which confers these additional properties. For example, the amino acid sequence of glutathione-S-transferase may be included or A non-C. perfringens antigenic protein may be included fused to the protein of the invention for the purpose of providing other immunity or labelling. Alternatively the polypeptide or protein of the invention may be in the form of a conjugate with another protein which confers such an additional desirable property.

The polypeptides of the invention may be prepared synthetically, or more suitably, they are obtained using recombinant DNA technology. Thus the invention further provides a nucleic acid which encodes a polypeptide as described above.

Suitably, the nucleic acid comprises the part of the sequence shown in SEQ ID No 5 which encodes the SEQ ID no 6.

Such nucleic acids may be incorporated into an expression vector, such as a plasmid, under the control of a promoter as understood in the art. The vector may include other structures as conventional in the art, such as signal sequences, leader sequences and enhancers, and can be used to transform a host cell, for example a prokaryotic cell such as *E. coli* or a eukaryotic cell. Transformed cells can then be cultured and polypeptide of the invention recovered therefrom, either from the cells or from the culture medium, depending upon whether the desired product is secreted from the cell or not.

In a further aspect of the invention there is provided a method for inducing an immune response protective against Clostridium perfringens epsilon toxin in a mammal, said method comprising administering to said mammal an polypeptide as described above.

Suitable mammals include humans and animals, such as sheep, lambs and goats.

The polypeptide may be administered to the mammal directly, for example in the form of a vaccine composition. Alternatively, a nucleic acid encoding it may be incorporated into a suitable vaccine vector, for example an attenuated live virus vaccine carrier under the control of suitable promoters etc. to ensure that the vector expresses the polypeptide *in situ*. Administration of the vector to the mammal thereby produces the desired immune response. Suitable vectors will be apparent to the skilled person. They may include vaccinia virus vectors, such as the Lister strain, or attenuated gut-colonising microorganisms such as attenuated strains of *Salmonella*.

In a further embodiment the present invention provides vaccine compositions comprising the polypeptides or proteins of the invention or as an alternative, a vector capable of expressing said polypeptide or protein, suitably in appropriate dosage units. The compositions are optionally complemented as necessary by further agents for optimising prot ction eg adjuvants and carriers, preferably pharmaceutically acceptable carriers and adjuvants. Freunds incomplete or complete adjuvant or alhydrogel may be used as typical

adjuvants, but other suitable candidates such as those described in WO 9203164 may be used. Carrier function may be fulfilled by saline solutions. The carrier may be one suited to parenteral administration, particularly intraperitoneal administration but optionally oral for example in a live vaccine vector such as an attenuated gut-colonising micro-organism, or administration in the form of droplets or capsules, such as liposomes or microcapsules as would be effective in delivering the composition to the airways of an individual for the purpose of evoking a mucosal immune response.

The microcapsule may comprise biodegradable polymers for example poly-lactic acid either with or without glycolic acid or with or without a block co-polymer which may contain the following repeat unit: $(POP-POE)_n$ where POP is polyoxypropylene and POE is polyoxyethylene. Block co-polymers which contain $(POP-POE)_n$ may be of particular use.

The proteins and fusion proteins of the present invention may be used as mucosal adjuvants. They may be co-administered with a non-C. perfringens antigenic protein - this may augment the mucosal immune response to the non-C. perfringens antigenic protein. There is evidence that epsilon toxin binds to a cell surface receptor in Payne et al 1994.

The invention will now be described with reference to the following diagrams and sequences by way of example only:

Figures

Figure 1 illustrates constructs of the present invention;

Sequences

SEQ ID No. 1 shows the nucleic acid sequence and corresponding amino acid sequence of the C. perfringens epsilon toxin gene. Amino acids -45 to -14 corresponds to the signal sequence. Amino acids -13 to -1 correspond to the prototoxin cleared by trypsin to produce active mature toxin. Amino acids 1-283 correspond to the mature toxin;

SEQ ID No. 2 shows the amino acids of SEQ ID NO. 1.

SEQ ID NO 3 represents the nucleic acid sequence and corresponding amino acid sequence of the mutated C. perfringens epsilon toxin gene in SDM10 wherein amino acid 106 of the mature toxin has been replaced by a proline;

SEQ ID NO 4 corresponds to amino acids of SEQ ID No 3.

SEQ ID No 5 represents the nucleic acid sequence of the C. perfringens epsilon toxin gene wherein the bases that code for amino acid 106 (bases 451-453) of the mature protein are represented by NNN; and

SEQ ID NO 6 represents the mature toxin part of the C. perfringens epsilon toxin gene wherein amino acid 106 is denoted Xaa indicating that this amino acid may be any amino acid except histidine.

Example 1

Production of Mutants

Mutants with single amino acid changes were constructed using oligonucleotide site directed mutagenesis. The epsilon toxin gene was supplied for site-directed mutagenesis in pBluescript II KS +/- and the mutated genes were delivered in this vector. The mutated gene was subcloned into pGEX3a, the epsilon toxin being expressed as a fusion with glutathione-S-transferase, and into pTrc99A, the epsilon toxin being expressed at high levels under the control of the trc promoter. These constructs are represented in Figure 1. The mutant with the mutation converting the histidine residue at position 106 to a proline is referred to as SDM10.

Example 2

Immunisation of mice

Groups of thirty mice were immunised intraperitoneally in Incomplete Freund's adjuvant (IFA) with epsilon toxoid, purified GST-epsilon fusion expressed by pGEXh1h2, purified GST-SDM10 fusion expressed by pGEXh1h2.10, or SDM10 periplasmic preparation expressed by pTrcEP7.10. Each dose was equivalent to 0.27nM of toxoid. Two control groups were included: mice immunised with 0.27nM GST (Sigma) in IFA and unimmunised mice. Mice were boosted on days 21 and 35. Ten mice per group were bled on day 48 and the sera were titred against recombinant epsilon toxin and epsilon toxin from C.perfringens 8346.

On day 54 the mice were challenged in groups of 6 mice with 10-10^3 LD_{50} doses of recombinant epsilon toxin, administered i.v. The mice were observed for 24h and times to death were noted.

Mice immunised with epsilon toxoid, GST-epsilon, GST-10 and SDM10 were fully protected against an intravenous challenge of up to 100 LD_{50} doses of toxin per mouse. All control mice died.

When the challenge dose was raised to 1000 LD_{50} per mouse, mice immunised with toxoid or with SDM10 survived. Five of six mice survived this level of challenge in the GST-10 and GST-epsilon groups.

Thus the polypeptides of the invention are as protective as the toxoid.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: The Secretary of State for Defence
(B) STREET: Defence Evaluation and Research Agency
(C) CITY: Ively Road
(D) STATE: Farnborough, Hants
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): GU14 0LX

(A) NAME: Richard William Titball
(B) STREET: CBD, Porton Down
(C) CITY: Salisbury
(D) STATE: Wilts.
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): SP4 0JQ

(A) NAME: Ethel Diane Williamson
(B) STREET: CBD, Porton Down
(C) CITY: Salisbury
(D) STATE: Wilts
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): SP4 0JQ

(A) NAME: Helen Louise Havard
(B) STREET: CBD, Porton Down
(C) CITY: Salisbury
(D) STATE: Wilts
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): SP4 0JQ

(A) NAME: Petra Claire Farquhar Oyston
(B) STREET: CBD, Porton Down
(C) CITY: Salisbury
(D) STATE: Wilts
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): SP4 0JQ

(A) NAME: Dean William Payne
(B) STREET: CBD, Porton Down
(C) CITY: Salisbury
(D) STATE: Wilts
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): SP4 0JQ

(ii) TITLE OF INVENTION: CLOSTRIDIUM PERFRINGENS VACCINES

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 987 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Clostridium perfringens

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 136..456

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..987

(ix) FEATURE:
(A) NAME/KEY: misc_signal
(B) LOCATION: 1..32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG AAA AAA AAT CTT GTA AAA AGT TTA GCA ATC GCA TCA GCG GTG ATA   48
Met Lys Lys Asn Leu Val Lys Ser Leu Ala Ile Ala Ser Ala Val Ile
-45                 -40                 -35                 -30

TCC ATC TAT TCA ATA GTT AAT ATT GTT TCA CCA ACT AAT GTA ATA GCT   96
Ser Ile Tyr Ser Ile Val Asn Ile Val Ser Pro Thr Asn Val Ile Ala
            -25                 -20                 -15

AAG GAA ATA TCT AAT ACA GTA TCT AAT GAA ATG TCC AAA AAA GCT TCT  144
Lys Glu Ile Ser Asn Thr Val Ser Asn Glu Met Ser Lys Lys Ala Ser
        -10                 -5                  1

TAT GAT AAT GTA GAT ACA TTA ATT GAG AAA GGA AGA TAT AAT ACA AAA  192
Tyr Asp Asn Val Asp Thr Leu Ile Glu Lys Gly Arg Tyr Asn Thr Lys
    5                   10                  15

TAT AAT TAC TTA AAG AGA ATG GAA AAA TAT TAT CCT AAT GCT ATG GCA  240
Tyr Asn Tyr Leu Lys Arg Met Glu Lys Tyr Tyr Pro Asn Ala Met Ala
20                  25                  30                  35

TAT TTT GAT AAG GTT ACT ATA AAT CCA CAA GGA AAT GAT TTT TAT ATT  288
Tyr Phe Asp Lys Val Thr Ile Asn Pro Gln Gly Asn Asp Phe Tyr Ile
                40                  45                  50

AAT AAT CCT AAA GTT GAA TTA GAT GGA GAA CCA TCA ATG AAT TAT CTT  336
Asn Asn Pro Lys Val Glu Leu Asp Gly Glu Pro Ser Met Asn Tyr Leu
            55                  60                  65
```

```
GAA GAT GTT TAT GTT GGA AAA GCT CTC TTA ACT AAT GAT ACT CAA CAA  384
Glu Asp Val Tyr Val Gly Lys Ala Leu Leu Thr Asn Asp Thr Gln Gln
          70                  75                  80

GAA CAA AAA TTA AAA TCA CAA TCA TTC ACT TGT AAA AAT ACT GAT ACA  432
Glu Gln Lys Leu Lys Ser Gln Ser Phe Thr Cys Lys Asn Thr Asp Thr
      85                  90                  95

GTA ACT GCA ACT ACT ACT CAT ACT GTG GGA ACT TCG ATA CAA GCA ACT  480
Val Thr Ala Thr Thr Thr His Thr Val Gly Thr Ser Ile Gln Ala Thr
100                 105                 110                 115

GCT AAG TTT ACT GTT CCT TTT AAT GAA ACA GGA GTA TCA TTA ACT ACT  528
Ala Lys Phe Thr Val Pro Phe Asn Glu Thr Gly Val Ser Leu Thr Thr
                120                 125                 130

AGT TAT AGT TTT GCA AAT ACA AAT ACA AAT ACT AAT TCA AAA GAA ATT  576
Ser Tyr Ser Phe Ala Asn Thr Asn Thr Asn Thr Asn Ser Lys Glu Ile
            135                 140                 145

ACT CAT AAT GTC CCT TCA CAA GAT ATA CTA GTA CCA GCT AAT ACT ACT  624
Thr His Asn Val Pro Ser Gln Asp Ile Leu Val Pro Ala Asn Thr Thr
        150                 155                 160

GTA GAA GTA ATA GCA TAT TTA AAA AAA GTT AAT GTT AAA GGA AAT GTA  672
Val Glu Val Ile Ala Tyr Leu Lys Lys Val Asn Val Lys Gly Asn Val
    165                 170                 175

AAG TTA GTA GGA CAA GTA AGT GGA AGT GAA TGG GGA GAG ATA CCT AGT  720
Lys Leu Val Gly Gln Val Ser Gly Ser Glu Trp Gly Glu Ile Pro Ser
180                 185                 190                 195

TAT TTA GCT TTT CCT AGG GAT GGT TAT AAA TTT AGT TTA TCG GAT ACA  768
Tyr Leu Ala Phe Pro Arg Asp Gly Tyr Lys Phe Ser Leu Ser Asp Thr
                200                 205                 210

GTA AAT AAG AGT GAT TTA AAT GAA GAT GGT ACT ATT AAT ATT AAT GGA  816
Val Asn Lys Ser Asp Leu Asn Glu Asp Gly Thr Ile Asn Ile Asn Gly
            215                 220                 225

AAA GGA AAT TAT AGT GCA GTT ATG GGA GAT GAG TTA ATA GTT AAG GTT  864
Lys Gly Asn Tyr Ser Ala Val Met Gly Asp Glu Leu Ile Val Lys Val
        230                 235                 240

AGA AAT TTA AAT ACA AAT AAT GTA CAA GAA TAT GTA ATA CCT GTA GAT  912
Arg Asn Leu Asn Thr Asn Asn Val Gln Glu Tyr Val Ile Pro Val Asp
    245                 250                 255

AAA AAA GAA AAA AGT AAT GAT TCA AAT ATA GTA AAA TAT AGG AGT CTT  960
Lys Lys Glu Lys Ser Asn Asp Ser Asn Ile Val Lys Tyr Arg Ser Leu
260                 265                 270                 275

TAT ATT AAG GCA CCA GGA ATA AAA TAA                             987
Tyr Ile Lys Ala Pro Gly Ile Lys  *
                280
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 329 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Lys Asn Leu Val Lys Ser Leu Ala Ile Ala Ser Ala Val Ile
-45 -40 -35 -30

Ser Ile Tyr Ser Ile Val Asn Ile Val Ser Pro Thr Asn Val Ile Ala
-25 -20 -15

Lys Glu Ile Ser Asn Thr Val Ser Asn Glu Met Ser Lys Lys Ala Ser
-10 -5 1

Tyr Asp Asn Val Asp Thr Leu Ile Glu Lys Gly Arg Tyr Asn Thr Lys
5 10 15

Tyr Asn Tyr Leu Lys Arg Met Glu Lys Tyr Tyr Pro Asn Ala Met Ala
20 25 30 35

Tyr Phe Asp Lys Val Thr Ile Asn Pro Gln Gly Asn Asp Phe Tyr Ile
40 45 50

Asn Asn Pro Lys Val Glu Leu Asp Gly Glu Pro Ser Met Asn Tyr Leu
55 60 65

Glu Asp Val Tyr Val Gly Lys Ala Leu Leu Thr Asn Asp Thr Gln Gln
70 75 80

Glu Gln Lys Leu Lys Ser Gln Ser Phe Thr Cys Lys Asn Thr Asp Thr
85 90 95

Val Thr Ala Thr Thr Thr His Thr Val Gly Thr Ser Ile Gln Ala Thr
100 105 110 115

Ala Lys Phe Thr Val Pro Phe Asn Glu Thr Gly Val Ser Leu Thr Thr
120 125 130

Ser Tyr Ser Phe Ala Asn Thr Asn Thr Asn Thr Asn Ser Lys Glu Ile
135 140 145

Thr His Asn Val Pro Ser Gln Asp Ile Leu Val Pro Ala Asn Thr Thr
150 155 160

Val Glu Val Ile Ala Tyr Leu Lys Lys Val Asn Val Lys Gly Asn Val
165 170 175

Lys Leu Val Gly Gln Val Ser Gly Ser Glu Trp Gly Glu Ile Pro Ser
180 185 190 195

Tyr Leu Ala Phe Pro Arg Asp Gly Tyr Lys Phe Ser Leu Ser Asp Thr
200 205 210

Val Asn Lys Ser Asp Leu Asn Glu Asp Gly Thr Ile Asn Ile Asn Gly
215 220 225

Lys Gly Asn Tyr Ser Ala Val Met Gly Asp Glu Leu Ile Val Lys Val
230 235 240

```
Arg Asn Leu Asn Thr Asn Asn Val Gln Glu Tyr Val Ile Pro Val Asp
245                 250                 255

Lys Lys Glu Lys Ser Asn Asp Ser Asn Ile Val Lys Tyr Arg Ser Leu
260                 265                 270                 275

Tyr Ile Lys Ala Pro Gly Ile Lys  *
                280
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 987 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Clostridium perfringens
(B) STRAIN: NCTC 8346

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: epsilon toxin gene

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 136..987

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..987

(ix) FEATURE:
(A) NAME/KEY: misc_signal
(B) LOCATION: 1..32

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 451..453

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG AAA AAA AAT CTT GTA AAA AGT TTA GCA ATC GCA TCA GCG GTG ATA   48
Met Lys Lys Asn Leu Val Lys Ser Leu Ala Ile Ala Ser Ala Val Ile
-45                 -40                 -35                 -30

TCC ATC TAT TCA ATA GTT AAT ATT GTT TCA CCA ACT AAT GTA ATA GCT   96
Ser Ile Tyr Ser Ile Val Asn Ile Val Ser Pro Thr Asn Val Ile Ala
                -25                 -20                 -15

AAG GAA ATA TCT AAT ACA GTA TCT AAT GAA ATG TCC AAA AAA GCT TCT  144
Lys Glu Ile Ser Asn Thr Val Ser Asn Glu Met Ser Lys Lys Ala Ser
            -10                 -5                  1
```

```
TAT GAT AAT GTA GAT ACA TTA ATT GAG AAA GGA AGA TAT AAT ACA AAA  192
Tyr Asp Asn Val Asp Thr Leu Ile Glu Lys Gly Arg Tyr Asn Thr Lys
  5                  10                  15

TAT AAT TAC TTA AAG AGA ATG GAA AAA TAT TAT CCT AAT GCT ATG GCA  240
Tyr Asn Tyr Leu Lys Arg Met Glu Lys Tyr Tyr Pro Asn Ala Met Ala
 20                  25                  30                  35

TAT TTT GAT AAG GTT ACT ATA AAT CCA CAA GGA AAT GAT TTT TAT ATT  288
Tyr Phe Asp Lys Val Thr Ile Asn Pro Gln Gly Asn Asp Phe Tyr Ile
             40                  45                  50

AAT AAT CCT AAA GTT GAA TTA GAT GGA GAA CCA TCA ATG AAT TAT CTT  336
Asn Asn Pro Lys Val Glu Leu Asp Gly Glu Pro Ser Met Asn Tyr Leu
         55                  60                  65

GAA GAT GTT TAT GTT GGA AAA GCT CTC TTA ACT AAT GAT ACT CAA CAA  384
Glu Asp Val Tyr Val Gly Lys Ala Leu Leu Thr Asn Asp Thr Gln Gln
     70                  75                  80

GAA CAA AAA TTA AAA TCA CAA TCA TTC ACT TGT AAA AAT ACT GAT ACA  432
Glu Gln Lys Leu Lys Ser Gln Ser Phe Thr Cys Lys Asn Thr Asp Thr
 85                  90                  95

GTA ACT GCA ACT ACT ACT CCG ACT GTG GGA ACT TCG ATA CAA GCA ACT  480
Val Thr Ala Thr Thr Thr Pro Thr Val Gly Thr Ser Ile Gln Ala Thr
100                 105                 110                 115

GCT AAG TTT ACT GTT CCT TTT AAT GAA ACA GGA GTA TCA TTA ACT ACT  528
Ala Lys Phe Thr Val Pro Phe Asn Glu Thr Gly Val Ser Leu Thr Thr
            120                 125                 130

AGT TAT AGT TTT GCA AAT ACA AAT ACA AAT ACT AAT TCA AAA GAA ATT  576
Ser Tyr Ser Phe Ala Asn Thr Asn Thr Asn Thr Asn Ser Lys Glu Ile
        135                 140                 145

ACT CAT AAT GTC CCT TCA CAA GAT ATA CTA GTA CCA GCT AAT ACT ACT  624
Thr His Asn Val Pro Ser Gln Asp Ile Leu Val Pro Ala Asn Thr Thr
    150                 155                 160

GTA GAA GTA ATA GCA TAT TTA AAA AAA GTT AAT GTT AAA GGA AAT GTA  672
Val Glu Val Ile Ala Tyr Leu Lys Lys Val Asn Val Lys Gly Asn Val
165                 170                 175

AAG TTA GTA GGA CAA GTA AGT GGA AGT GAA TGG GGA GAG ATA CCT AGT  720
Lys Leu Val Gly Gln Val Ser Gly Ser Glu Trp Gly Glu Ile Pro Ser
180                 185                 190                 195

TAT TTA GCT TTT CCT AGG GAT GGT TAT AAA TTT AGT TTA TCG GAT ACA  768
Tyr Leu Ala Phe Pro Arg Asp Gly Tyr Lys Phe Ser Leu Ser Asp Thr
            200                 205                 210

GTA AAT AAG AGT GAT TTA AAT GAA GAT GGT ACT ATT AAT ATT AAT GGA  816
Val Asn Lys Ser Asp Leu Asn Glu Asp Gly Thr Ile Asn Ile Asn Gly
        215                 220                 225

AAA GGA AAT TAT AGT GCA GTT ATG GGA GAT GAG TTA ATA GTT AAG GTT  864
Lys Gly Asn Tyr Ser Ala Val Met Gly Asp Glu Leu Ile Val Lys Val
    230                 235                 240
```

```
AGA AAT TTA AAT ACA AAT AAT GTA CAA GAA TAT GTA ATA CCT GTA GAT  912
Arg Asn Leu Asn Thr Asn Asn Val Gln Glu Tyr Val Ile Pro Val Asp
    245                 250                 255

AAA AAA GAA AAA AGT AAT GAT TCA AAT ATA GTA AAA TAT AGG AGT CTT  960
Lys Lys Glu Lys Ser Asn Asp Ser Asn Ile Val Lys Tyr Arg Ser Leu
260                 265                 270                 275

TAT ATT AAG GCA CCA GGA ATA AAA TAA                              987
Tyr Ile Lys Ala Pro Gly Ile Lys  *
                280
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 329 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 106

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Lys Lys Asn Leu Val Lys Ser Leu Ala Ile Ala Ser Ala Val Ile
-45                 -40                 -35                 -30

Ser Ile Tyr Ser Ile Val Asn Ile Val Ser Pro Thr Asn Val Ile Ala
                -25                 -20                 -15

Lys Glu Ile Ser Asn Thr Val Ser Asn Glu Met Ser Lys Lys Ala Ser
            -10                 -5                  1

Tyr Asp Asn Val Asp Thr Leu Ile Glu Lys Gly Arg Tyr Asn Thr Lys
        5                   10                  15

Tyr Asn Tyr Leu Lys Arg Met Glu Lys Tyr Tyr Pro Asn Ala Met Ala
    20                  25                  30                  35

Tyr Phe Asp Lys Val Thr Ile Asn Pro Gln Gly Asn Asp Phe Tyr Ile
                40                  45                  50

Asn Asn Pro Lys Val Glu Leu Asp Gly Glu Pro Ser Met Asn Tyr Leu
            55                  60                  65

Glu Asp Val Tyr Val Gly Lys Ala Leu Leu Thr Asn Asp Thr Gln Gln
        70                  75                  80

Glu Gln Lys Leu Lys Ser Gln Ser Phe Thr Cys Lys Asn Thr Asp Thr
    85                  90                  95

Val Thr Ala Thr Thr Thr Pro Thr Val Gly Thr Ser Ile Gln Ala Thr
100                 105                 110                 115

Ala Lys Phe Thr Val Pro Phe Asn Glu Thr Gly Val Ser Leu Thr Thr
                120                 125                 130
```

Ser Tyr Ser Phe Ala Asn Thr Asn Thr Asn Thr Asn Ser Lys Glu Ile
135 140 145

Thr His Asn Val Pro Ser Gln Asp Ile Leu Val Pro Ala Asn Thr Thr
150 155 160

Val Glu Val Ile Ala Tyr Leu Lys Lys Val Asn Val Lys Gly Asn Val
165 170 175

Lys Leu Val Gly Gln Val Ser Gly Ser Glu Trp Gly Glu Ile Pro Ser
180 185 190 195

Tyr Leu Ala Phe Pro Arg Asp Gly Tyr Lys Phe Ser Leu Ser Asp Thr
200 205 210

Val Asn Lys Ser Asp Leu Asn Glu Asp Gly Thr Ile Asn Ile Asn Gly
215 220 225

Lys Gly Asn Tyr Ser Ala Val Met Gly Asp Glu Leu Ile Val Lys Val
230 235 240

Arg Asn Leu Asn Thr Asn Asn Val Gln Glu Tyr Val Ile Pro Val Asp
245 250 255

Lys Lys Glu Lys Ser Asn Asp Ser Asn Ile Val Lys Tyr Arg Ser Leu
260 265 270 275

Tyr Ile Lys Ala Pro Gly Ile Lys *
280

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 987 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Clostridium perfringens

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 136..987

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..987

(ix) FEATURE:
(A) NAME/KEY: misc_signal
(B) LOCATION: 1..32

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION: 451..453

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGAAAAAAA ATCTTGTAAA AAGTTTAGCA ATCGCATCAG CGGTGATATC CATCTATTCA 60

ATAGTTAATA TTGTTTCACC AACTAATGTA ATAGCTAAGG AAATATCTAA TACAGTATCT 120

AATGAAATGT CCAAAAAAGC TTCTTATGAT AATGTAGATA CATTAATTGA GAAAGGAAGA 180

TATAATACAA AATATAATTA CTTAAAGAGA ATGGAAAAAT ATTATCCTAA TGCTATGGCA 240

TATTTTGATA AGGTTACTAT AAATCCACAA GGAAATGATT TTTATATTAA TAATCCTAAA 300

GTTGAATTAG ATGGAGAACC ATCAATGAAT TATCTTGAAG ATGTTTATGT TGGAAAAGCT 360

CTCTTAACTA ATGATACTCA ACAAGAACAA AAATTAAAAT CACAATCATT CACTTGTAAA 420

AATACTGATA CAGTAACTGC AACTACTACT NNNACTGTGG GAACTTCGAT ACAAGCAACT 480

GCTAAGTTTA CTGTTCCTTT TAATGAAACA GGAGTATCAT TAACTACTAG TTATAGTTTT 540

GCAAATACAA ATACAAATAC TAATTCAAAA GAAATTACTC ATAATGTCCC TTCACAAGAT 600

ATACTAGTAC CAGCTAATAC TACTGTAGAA GTAATAGCAT ATTTAAAAAA AGTTAATGTT 660

AAAGGAAATG TAAAGTTAGT AGGACAAGTA AGTGGAAGTG AATGGGGAGA GATACCTAGT 720

TATTTAGCTT TTCCTAGGGA TGGTTATAAA TTTAGTTTAT CGGATACAGT AAATAAGAGT 780

GATTTAAATG AAGATGGTAC TATTAATATT AATGGAAAAG GAAATTATAG TGCAGTTATG 840

GGAGATGAGT TAATAGTTAA GGTTAGAAAT TTAAATACAA ATAATGTACA AGAATATGTA 900

ATACCTGTAG ATAAAAAAGA AAAAAGTAAT GATTCAAATA TAGTAAAATA TAGGAGTCTT 960

TATATTAAGG CACCAGGAAT AAAATAA 987

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 283 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Clostridium perfringens

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 106

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Lys Ala Ser Tyr Asp Asn Val Asp Thr Leu Ile Glu Lys Gly Arg Tyr
1 5 10 15

Asn Thr Lys Tyr Asn Tyr Leu Lys Arg Met Glu Lys Tyr Tyr Pro Asn
20 25 30

Ala Met Ala Tyr Phe Asp Lys Val Thr Ile Asn Pro Gln Gly Asn Asp
35 40 45

Phe Tyr Ile Asn Asn Pro Lys Val Glu Leu Asp Gly Glu Pro Ser Met
50 55 60

Asn Tyr Leu Glu Asp Val Tyr Val Gly Lys Ala Leu Leu Thr Asn Asp
65 70 75 80

Thr Gln Gln Glu Gln Lys Leu Lys Ser Gln Ser Phe Thr Cys Lys Asn
85 90 95

Thr Asp Thr Val Thr Ala Thr Thr Thr Xaa Thr Val Gly Thr Ser Ile
100 105 110

Gln Ala Thr Ala Lys Phe Thr Val Pro Phe Asn Glu Thr Gly Val Ser
115 120 125

Leu Thr Thr Ser Tyr Ser Phe Ala Asn Thr Asn Thr Asn Thr Asn Ser
130 135 140

Lys Glu Ile Thr His Asn Val Pro Ser Gln Asp Ile Leu Val Pro Ala
145 150 155 160

Asn Thr Thr Val Glu Val Ile Ala Tyr Leu Lys Lys Val Asn Val Lys
165 170 175

Gly Asn Val Lys Leu Val Gly Gln Val Ser Gly Ser Glu Trp Gly Glu
180 185 190

Ile Pro Ser Tyr Leu Ala Phe Pro Arg Asp Gly Tyr Lys Phe Ser Leu
195 200 205

Ser Asp Thr Val Asn Lys Ser Asp Leu Asn Glu Asp Gly Thr Ile Asn
210 215 220

Ile Asn Gly Lys Gly Asn Tyr Ser Ala Val Met Gly Asp Glu Leu Ile
225 230 235 240

Val Lys Val Arg Asn Leu Asn Thr Asn Asn Val Gln Glu Tyr Val Ile
245 250 255

Pro Val Asp Lys Lys Glu Lys Ser Asn Asp Ser Asn Ile Val Lys Tyr
260 265 270

Arg Ser Leu Tyr Ile Lys Ala Pro Gly Ile Lys
275 280

Claims

1. A polypeptide capable of producing an immune response which is protective against *Clostridium perfringens*, said polypeptide comprising an amino acid sequence which has at least 60% homology with the amino acid sequence of Clostridium perfringens epsilon toxin or an immunogenic fragment thereof, characterised in that the amino acid residue corresponding to residue 106 of the mature toxin is of an amino acid other than histidine.

2. A polypeptide according to claim 1 which comprises an amino acid sequence which has at least 90% homology with the amino acid sequence of Clostridium perfringens epsilon toxin or an immunogenic fragment thereof.

3. A polypeptide according to claim 1 or claim 2 wherein the amino acid residue corresponding to residue 106 of the mature toxin is a non polar amino acid.

4. A polypeptide according to claim 3 wherein the wherein the amino acid residue corresponding to residue 106 of the mature toxin is proline.

5. A polypeptide according to claim 5 comprising an amino acid sequence as shown in SEQ ID No 4.

6. A polypeptide according to any one of the preceding claims which is fused to a further amino acid sequence.

7. A polypeptide according to claim 6 wherein said further amino acid sequence comprises glutathione-S-transferase.

8. A polypeptide according to any one of the preceding claims which is conjugated to another protein.

9. A nucleic acid which encodes a polypeptide as claimed in any one of claims 1 to 7.

10. A nucleic acid according to claim 9 which comprises at least the part of the sequence shown in SEQ ID No 5 which encodes the SEQ ID no 6.

11. A nucleic acid according to claim 9 which comprises SEQ ID No 5.

12. An expression vector which comprises a nucleic acid according to any one of claims 9 to 11.

13. A cell transformed with an expression vector according to claim 12.

14. A process for producing a polypeptide according to any one of claims 1 to 8 which method comprises culturing a cell according to claim 13 and recovering polypeptide therefrom or from the culture medium thereof.

15. A vaccine composition comprising a polypeptide according to any one of claims 1- 8 together with a pharmaceutically acceptable carrier.

16. A vaccine composition as claimed in claim 14 further comprising an adjuvant.

17. A vaccine composition as claimed in claim 15 wherein the adjuvant is Freunds incomplete adjuvant or an aluminium salt.

18. A plasmid comprising recombinant DNA encoding for a polypeptide as claimed in any one of claims 1-7.

19. A polypeptide as claimed in any one of claims 1-8 for use in the preparation of a medicament.

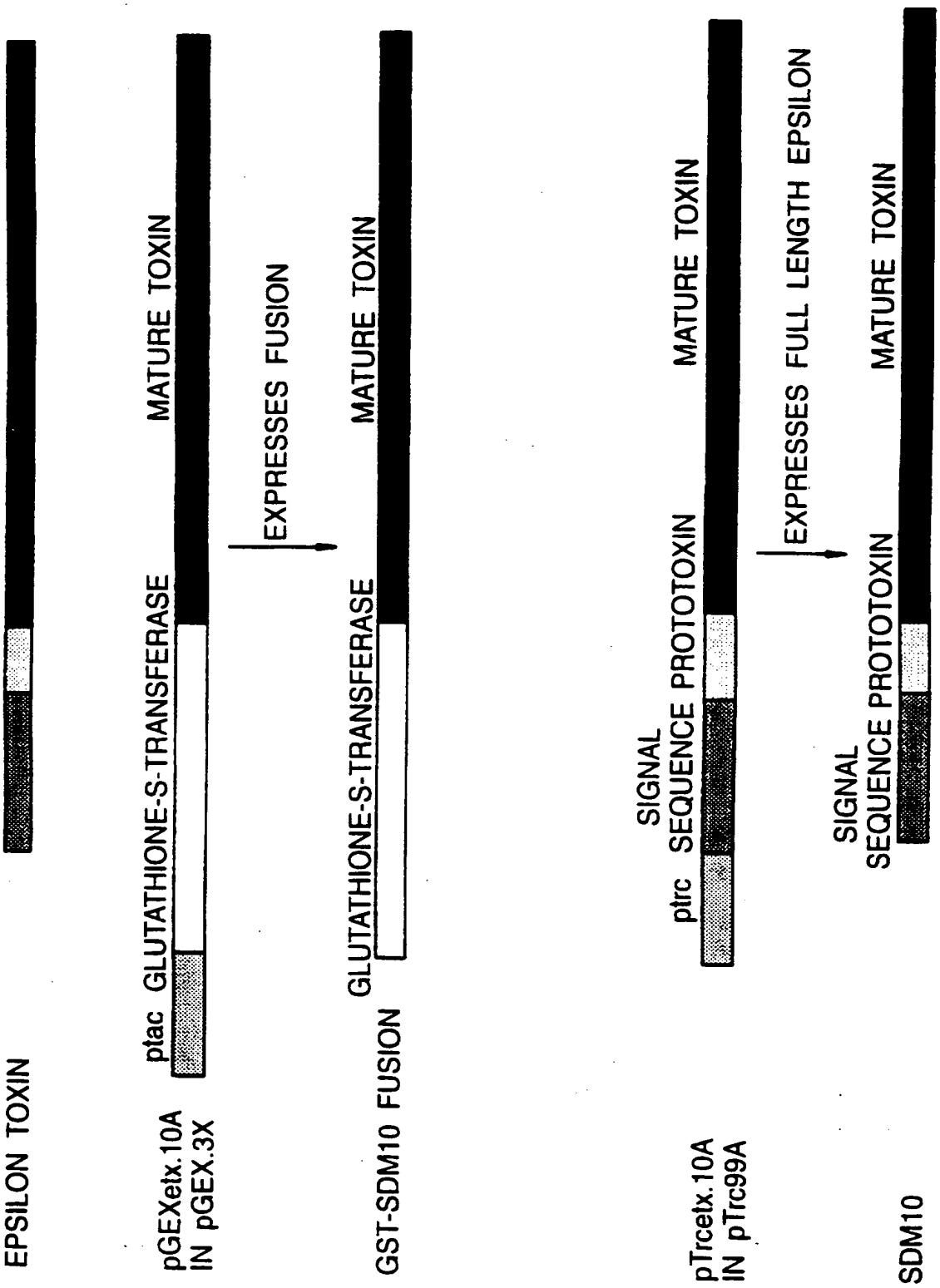
20. A vaccine composition comprising a virus vector which comprises a nucleic acid according to any one of claims 9 to 11.

21. A mucosal adjuvant comprising the protein or fusion protein of any of claims 1-8.

22. A method for inducing an immune response protective against Clostridium perfringens epsilon toxin in a mammal, said method comprising administering to said mammal an polypeptide as claimed in any one of claims 1 to 8.

23. A method according to claim 22 wherein the mammal is a sheep, lamb or goat.

Fig.1.



INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/00660

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C12N15/62 C07K14/33 A61K39/08 C12N15/86
C12N1/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	INFECTION AND IMMUNITY, vol. 60, no. 1, January 1992, pages 102-110, XP000674523 HUNTER S.E. ET AL.: "Cloning and nucleotide sequencing of the Clostridium perfringens epsilon-toxin gene and its expression in Escherichia coli." cited in the application see the whole document ---	1-23
A	FEMS MICROBIOLOGY LETTERS, vol. 41, 1987, pages 317-319, XP000674521 SAKURAI J. AND NAGAHAMA M.: "Histidine residues in Clostridium perfringens epsilon toxin." cited in the application see the whole document ---	1-23

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 June 1997	01.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Mandl, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/GB 97/00660

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 895 073 A (WELLCOME FOUND. LTD.) 2 May 1962 see the whole document ---	15-17, 19-23
A	FEMS MICROBIOLOGY LETTERS, vol. 68, 1990, pages 261-265, XP000674531 TITBALL R.W. AND RUBIDGE T.: "The role of histidine residues in the alpha toxin of Clostridium perfringens." see the whole document -----	1-23

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/00660

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 20,23
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 22 and 23 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No
PCT/GB 97/00660

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 895073 A		NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)